Remarks

Currently, claims 64-88, including independent claim 64, are pending in the present application. Independent claim 64, for example, is directed to a method for detecting an analyte within a test sample. The method comprises providing a lateral flow assay device that comprises a porous membrane in fluid communication with phosphorescent particles conjugated with a specific binding member. The phosphorescent particles comprise a phosphorescent label encapsulated within a matrix, the phosphorescent label having an emission lifetime of about 1 microsecond or more. The porous membrane defines a detection zone within which is immobilized a capture reagent. The lateral flow assay device is contacted with the test sample. The detection zone is subjected to pulses of illumination to generate a detection signal. The intensity of the detection signal is measured. The amount of the analyte within the test sample is proportional to the intensity of the detection signal.

In the Office Action, independent claim 64 was rejected under *35 U.S.C. §103(a)* as being obvious over Daniels, et al. (U.S. Patent Application Publication No. 2002/0004246) in view of O'Riordan, et al. (*Anal. Chem.,* **74** (2002) 5845-5850) and further in view of Klimant (U.S. Patent No. 6,770,220). Daniels, et al. is directed to a method for detecting an analyte. More specifically, the method employs semiconductor nanocrystals and microspheres dyed with semiconductor nanocrystals as detectable labels in a variety of biological and chemical formats, including immunochromatographic strip assays. As correctly noted by the Examiner, however, Daniels, et al. fails to disclose one or more limitations of the present claims. For example, Daniels, et al. does not disclose the use of phosphorescent particles that comprise a phosphorescent label

encapsulated within a matrix, the phosphorescent label having an emission lifetime of about 1 microsecond or more.

Nevertheless, O'Riordan, et al. was cited in combination with Daniels, et al. More specifically, the Office Action cited O'Riordan, et al. for the teaching of a solid-phase immunoassay utilizing phosphorescent porphyrin labels that display high quantum yields, long phosphorescent lifetimes, and intense absorption bands.

Applicants respectfully submit that no teaching or motivation exists to combine the immunonochromatographic strip assays of Daniels, et al. with the phosphorescent porphyrin labels of O'Riordan, et al. to arrive at the limitations of independent claim 64. Though both references do utilize luminescent materials, the semiconductor nanocrystals of Daniels, et al. and the metalloporphyrins of O'Riordan, et al. are quite different with regard to many characteristics including luminescent properties, as pointed out in the previous response dated September 18, 2006. Additional differences exist between the labeling materials employed in the two references as well. For instance, the semiconductor nanocrystals of Daniels, et al. are inorganic crystallite *particles* having a size of between about 1nm and about 1000nm (paragraph [0079]). These particles can be either directly or indirectly linked, conjugated to or associated with a specific-binding molecule (paragraph [0098]).

In contrast, O'Riordan, et al. utilizes a monofunctional labeling reagent of platinum (II) coproporphyrin-I with isothiocyanate reactive group (PtCP-NCS). This is a *molecular* label, not a particle label as is utilized in Daniels, et al. Not only are these two types of labels fundamentally different in terms of optical, chemical, and physical properties, Daniels, et al. actually teaches against utilization of molecular tags in the

form of organic fluorescent dyes due to such problems as photo-bleaching (paragraph [0012]), spectral overlap (paragraph [0013]), and a general lack of robustness (paragraph [0018]). Given the negative teaching of Daniels, et al. with regard to molecular tags, one of ordinary skill in the art would simply not find motivation to combine the teachings of Daniels, et al. with a reference that is directed to the utilization of molecular tags, such as O'Riordan, et al.

Additional differences exist between the references as well. For instance, the system of O'Riordan, et al. is a solution-based chemistry system rather than a dry chemistry system as is found in Daniels, et al. The solution-based chemistry of O'Riordan, et al. is utilized at least for the reason that oxygen is known to quench the phosphorescence of porphyrin labels (page 5846, second column, paragraph labeled 'Spectral Measurements'). O'Riordan, et al., in order to take advantage of strong phosphorescence, requires the addition of a chemical deoxygenator (e.g., Na_2SO_3, pg. 5846, bottom of the second column). Addition of a chemical deoxygenator will only be practical for the removal of oxygen in a solution-based system. In a dry system, much of the phosphorescence of a molecular phosphorescent label would be quenched by oxygen unless the assay were to be carried out under oxygen-free conditions, such as vacuum, which is not practical in many circumstances.

According to the process of O'Riordan, et al., the monofunctional PtCP-NCS reagent is labeled with a protein or biotin according to a solution-based procedure (page 5846, paragraphs entitled 'Labeling of Proteins with PtCP-NCS' and 'Labeling of Biotin'). The assay itself is similarly carried out in solution. Specifically, AFP is immobilized on a solid phase, i.e., the wells of microtiter plates, via a monoclonal antihuman AFP

antibody (page 5847, first paragraph). Following immobilization of the AFP, the sandwich assay is completed by addition to the wells of 100μL of a 20 nM concentration of secondary antibody PtCP conjugate, monoclonal antihuman AFP antibody. This solution is incubated for 1 hour at 37°C (page 5847, second paragraph). Other immunoassay schemes described include multiple incubation times of 1 hour each upon addition of each successive reagent. Moreover, O'Riordan, et al. teaches that such 1 hour incubation times are not typical, and to obtain acceptable labeling, incubation times of six hours or greater are typically required (page 5847, paragraph labeled 'Results and Discussion').

Following incubation, the plates are washed and the bound conjugate is desorbed into solution and further incubated. In addition, phosphate-sulfite solution is added to remove oxygen, which is done in order to prevent quenching of the phosphorescence of the porphyrin labels. On the final page of O'Riordan, et al., the authors state that this final label desorption step can be eliminated. In this case, the final conjugated product could be detected directly from the solid phase, though as pointed out in the article, this would lead to a decrease in sensitivity (page 5850, first column, second paragraph). This decrease in sensitivity would be due to the quenching of the phosphorescent label in the presence of oxygen.

Even should the final desorption step be eliminated, the assay reactions of O'Riordan, et al. are still carried out in solution over a long incubation period. There is no suggestion, teaching or motivation to suggest that the molecular phosphorescent labels of O'Riordan, et al., which are quenched by oxygen, could be utilized in a dry chemistry system such as that of Daniels, et al. Moreover, the lateral flow system of

Daniels, et al. is not conducive to the long incubation periods necessary for the assay reagents of O'Riordan, et al. One of ordinary skill in the art would simply not be motivated to attempt to utilize the molecular phosphorescent labels of O'Riordan, et al. in place of the semiconductor nanocrystals and microspheres of the lateral flow system of Daniels, et al.

In the Office Action, it was stated that O'Riordan, et al. teaches that the particles and phosphorescent system of O'Riordan, et al. will work with a solid surface, such as the membrane surface of Daniels, et al. While O'Rioradan, et al. does describe a solid-phase immunoassay, this does not imply that the system of O'Riordan, et al. is similar to that of Daniels, et al. As discussed above, O'Riordan, et al. does not teach particles, but rather molecular labels. In addition, while O'Riordan, et al. does teach that the final desorption step of the solid phase immunoassay process may be eliminated, and the system is suitable for TR luminescence measurements from solid surfaces, O'Riordan, et al. does not teach or suggest that the immunoassay can be carried out in a dry chemistry system such as that of Daniels, et al. Solid phase immunoassay is the adaptation of the antigen-antibody reaction for the quantification of an analyte. In a solid phase immunoassay, one component of the assay, either the antigen or the antibody, is immobilized on a solid support. In a competitive-type solid phase immunoassay system, the analyte is then allowed to compete for the available binding sites with a known amount of a labeled analyte molecule. The term 'solid phase immunoassay' in no way implies that the immunoassay is carried out in a dry chemistry system. It simply means that one of the components is immobilized on a solid support. The solution chemistry system of O'Riordan, et al. is a solid phase immunoassay in

which the assay reactions take place in an oxygen-free environment (i.e., solution) and the phosphorescence of the molecular labels are maintained throughout the process.

Applicant respectfully submits that there is no motivation to incorporate the molecular PtCP-NCS labeling reagent of O'Riordan, et al., which uses the molecular labels for time-resolved detection in a solution-based chemistry assay system, with the devices of Daniels, et al., in the specific manner required by independent claim 64. Porous membranes present a wide variety of problems for time-resolved phosphorescent detection. For example, many membranes, such as nitrocellulose membranes, exhibit strong fluorescence when excited in the UV and visible regions. This fluorescence may interfere with the accuracy of phosphorescence measurements, and in particular with detection during the emission lifetime of a phosphorescent label, as is required by independent claim 64. Such problems and conditions are neither encompassed nor addressed in the cited references. In light of the above, Applicant respectfully submits that one of ordinary skill in the art would not have found it obvious to make the combination proposed in the Office Action and that no reasonable expectation of success would have existed to make the combination proposed in the Office Action.

Notwithstanding, the combination of O'Riordan, et al. and Daniels, et al. still fails to disclose phosphorescent particles comprising a phosphorescent label encapsulated within a matrix as is found in independent claim 64. Thus, the Office Action cited Klimant in combination with O'Riordan, et al. and Daniels, et al. in an attempt to render obvious independent claim 64. Specifically, Klimant was cited as teaching the

production and use of luminescent microparticles wherein the phosphorescent labels are incorporated within solid particles.

Applicant notes, however, that even should one attempt to combine O'Riordan, et al. with Daniels, et al., absent proper motivation to do so, no motivation would have existed to combine the teachings of O'Riordan, et al. with the teachings of Klimant as suggested in the Office Action. Klimant is directed to the incorporation of phosphorescent substances into a solid matrix to shield them from interfering substances (e.g., O_2). In stark contrast, the phosphorescent labels of O'Riordan, et al. are not encapsulated, but instead simply mixed with Na_2SO_3, as discussed above, to eliminate interference by molecular oxygen and other quenchers. The molecular labels of O'Riordan, et al. form conjugates with proteins, biotin, etc. and participate in the immunoassay through the further association of the conjugate with AFP to form sandwich assays. Due to the substantial differences in the fundamental construction and operation of the phosphorescent systems, one of ordinary skill in art would simply not have selectively picked and chosen certain aspects of O'Riordan, et al. for incorporation into Klimant.

Applicant emphasizes that the issue in conducting an analysis under *35 U.S.C. § 103(a)* is not whether a theoretical re-design of a device is *possible* or that it might be *obvious to try* the modification. Instead, the issue hinges on whether the claimed invention as a whole would have been obvious. In this case, the Office Action parsed and dissected only certain portions of Klimant and O'Riordan, et al., and then used these dissected portions in a way that would require a substantial reconstruction of Daniels, et al. Clearly, the Office Action is using the present application as a "blueprint"

for selectively re-designing the references, which is improper under *35 U.S.C. § 103*. Thus, for at least the reasons set forth above, Applicant respectfully submits that one of ordinary skill in the art would not have found it obvious to modify the references in the manner suggested in the Office Action.

Applicants also respectfully submit that for at least the reasons indicated above relating to corresponding independent claim 64, the pending dependent claims patentably define over the references cited. However, Applicants also note that the patentability of the dependent claims certainly does not hinge on the patentability of independent claim 64. In particular, it is believed that some or all of these claims may possess features that are independently patentable, regardless of the patentability of the independent claims. For example, none of the cited references disclose or suggest a method for detecting an analyte within a test sample comprising providing a lateral flow assay device that comprises a porous membrane, the porous membrane defining a calibration zone within which is immobilized a capture reagent comprising a polyelectrolyte as is found in dependent claim 92.

It is believed that the present application is in complete condition for allowance and favorable action, therefore, is respectfully requested. Examiner DiRamio is invited and encouraged to telephone the undersigned, however, should any issues remain after consideration of this Amendment.

Please charge any additional fees required by this Amendment to Deposit Account No. 04-1403.

Respectfully requested,

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